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Characterization of nivolumab associated skin reactions in patients with metastatic non-small cell lung cancer

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Brief Report

Characterization of nivolumab associated skin reactions in patients with metastatic non-small cell lung cancer

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Abstract

Immune checkpoint inhibitors have led to considerable therapy improvement in cancer patients. Autoimmune side effects including skin reactions are frequently observed. In melanoma those include rash and vitiligo and were shown to be associated with a prolonged overall survival. Little is known about skin reactions in NSCLC patients during immunotherapy.

Here, we retrospectively investigated immune-related adverse skin reactions (irAEs) in 40 patients with metastatic non-small cell lung cancer (NSCLC) treated with the anti PD-1 antibody nivolumab. 7 out of 40 patients (17%) developed an irAEs.

Skin irAEs correlated with tumor responses in 5 of 12 responders (42%) as compared to 2 of 27 non-responders (7%). Histologically, scaly plaques showed dermatitis consisting mainly of lymphocytes.

We observed a positive correlation between skin irAEs and tumor responses in patients with NSCLC treated with nivolumab. Patterns of lymphocytic skin infiltration differed depending on the histological tumor subtype (adenocarcinoma versus squamous cell carcinoma NSCLC).

Key words

immunotherapy, anti-PD1, non-small cell lung cancer, dermatology, pathology

Introduction

Non-small cell lung cancer (NSCLC) accounts for approximately 85% of all lung cancers and can be divided into two major histological subtypes: adenocarcinoma (AC) and squamous cell carcinoma (SCC). Standard first-line treatment of metastatic disease consists of platinum-based chemotherapy. In selected molecular-defined subgroups the first-line therapy is a targeted therapy using tyrosine kinase inhibitors (TKI). [1]

The introduction of immunotherapy has significantly improved therapy outcome. Two phase III trials with the anti-PD1 (anti-programmed death 1 receptor) antibody nivolumab led to its approval in 2015 after it had been approved for metastatic melanoma in 2014. [2-5]

PD1-checkpoint inhibitors block the interaction between PD1-receptors on T-cells and their ligand PD-L1. Blocking PD1-receptors enhances T-cell response against cancer cells. [6] In turn, activated T-cells can cause autoimmune-mediated side effects, such as skin rash, colitis, hepatitis or pneumonitis. These are generally manageable and reversible in most cases. Rash and pruritus are frequent immune-related dermatologic adverse reactions (skin irAEs) during immunotherapy, which have been reported to occur in up to 25 % of melanoma patients. [4, 5, 7, 8] Toxicity data for anti-PD1 approval in NSCLC reports skin irAEs in approximately 10% of patients [2, 3, 9], but as of recently higher rates have emerged. [10, 11] Grading of toxicity is commonly classified according to Common Terminology Criteria for Adverse Events (CTCAE version 4.0). Management of skin irAEs includes symptomatic treatment with antihistamines and topical steroids, but may be complemented with systemic immunosuppression in severe cases. [12] Previous data strongly suggest that skin

irAEs under anti-PD1 therapy are associated with increased overall survival (OS) and may serve as a parameter to predict a better therapy response. [13, 14]

The aim of this study is to i) characterize skin irAEs in patients with advanced lung cancer treated with nivolumab and ii) to specify the pattern of T-cell infiltration in biopsies of these skin lesions.

Materials and Methods

Assessing tumor response to therapy with nivolumab and skin irAEs

After approval by the local ethics committee (EKSG Nr. 16/059) and in accordance with the Declaration of Helsinki Principles, we retrospectively analyzed patients with metastatic NSCLC treated with nivolumab (Opdivo®, Bristol-Meyers Squibb, SA). Treatment was initiated between January 2015 and February 2016 within an early access program at the Cantonal Hospital St. Gallen (Switzerland). Patients had at least one treatment cycle with the standard dose of 3 mg/kg i.v. over 60 minutes every two weeks. Tumor response was evaluated using computed tomography (CT) and was categorized as progressive disease (PD), stable disease (SD) and partial remission (PR) according to RECIST criteria 1.1. [16] We further assessed results of skin examinations that had been performed during patient visits and classified skin irAEs according to clinical dermatological criteria.

Immunohistochemistry analysis

Histology of punch biopsies from untreated skin rash was available from 4 patients, of which 2 had SCC and 2 had AC. Biopsies of the SCC patients were taken from the right forearm and right lower limb and of the AC patients from the right cheek and left lower limb (one each, respectively). All punch biopsies had been performed by a dermatologist. Histopathological analyses were conducted independently by a pathologist and a dermatopathologist. From each representative paraffin-embedded

skin block four microns-thick sections were obtained for HE and immunohistochemistry (IHC) staining (CD3, CD4 and CD8). For each sample we determined lymphocyte counts in the epidermis and dermis per 1mm^2 (= 4 random high power fields).

Results

Tumor response and skin irAEs in NSCLC under nivolumab therapy

We identified 41 patients treated with at least one single dose of nivolumab. One patient was excluded because consent was refused. Patient age ranged from 46 to 88 years (mean 65 years). 22 (55%) were male and 18 (45%) female (Table 1). On average, the 40 patients were treated with 7 cycles, ranging from 1 to 25 cycles. The interval between treatment initiation and appearance of a skin irAE was 3 cycles.

23 (57%) of our patients had ACs, 14 (35%) SCCs and in 3 (8%) the tumor displayed features of both subtypes (mixed subtype). 27 (67%) showed disease progression under nivolumab therapy. We were unable to assess disease progression in one individual because the patient had died due to ileus before a re-staging could be performed.

7 (17%) developed a skin irAE under treatment. All skin reactions were classified grade I or II (CTCAE 4.0). In this subpopulation 4 (57%) were scaly plaques (Figure 1A/B) and 3 (43%) intense pruritus without visible skin lesions. Only 2 (29%) with skin irAEs had PD, as opposed to all patients, where the majority had PD. Furthermore, skin irAEs correlated with tumor responses in 5 of 12 responders (42%) as compared to 2 of 27 non-responders (7%).

Immune infiltrate patterns of skin rash under nivolumab therapy correlates with NSCLC subtype

Immunohistochemistry of the skin biopsies showed that the distribution of CD3+ T-cells in the inflammatory skin infiltrate was dependent on the cancer subtype (Figure 1F/G). We observed an analogous cell distribution pattern among cytotoxic CD8+ T-cells. In patients with SCCs the lymphocyte infiltrates were more prominent above the basal cell membrane whereas patients with ACs showed CD8+ T-cell infiltrates more accentuated towards the dermis (Figure 2A-D). Quantification of the T-cell infiltrates further supported this observation: the ratios of CD8+ T-cells found in the epidermis compared to all CD8+ T-cells of two patients with SCCs were 22% and 44%, respectively, while in two patients with ACs the ratios were 3% and 10%, respectively (Figure 2A-D).

Discussion

Autoimmune-mediated side effects affecting different organs are known to occur during treatment with immunotherapy. In NSCLC patients treated with nivolumab skin eruptions and pruritus have been reported in approximately 10% or higher. [2, 3, 9-11] This was also reflected by our study. Several authors reported higher numbers of skin irAEs in melanoma patients under nivolumab treatment. [4, 5, 7, 8]

An association between appearance of skin irAEs and overall survival in melanoma has been reported. [13-15, 17] Similarly, our data suggests an association between skin irAEs and tumor response. The histological analysis of the rashes on the one hand allowed us to rule out clinical differential diagnoses of eczema and psoriasis and on the other hand revealed that the rashes were characterized by an inflammation rich in CD3+ T-lymphocytes. Immunophenotypisation showed analogous lichenoid patterns among rashes with SCC-patients. They displayed distinct epidermotropic

inflammatory infiltrates consisting mainly of cytotoxic CD8+ T-cells. Civatte bodies (damaged basal keratinocytes) suggest that these lymphocytes induce keratinocyte death. From these findings we deduct that the skin of these patients harbored auto-immune T-cells that were activated and attacked healthy keratinocytes. It is further possible that tumor-specific T-cells, activated by anti-PD1 treatment, migrated to the skin. Since T-cells can migrate and scan antigens presented by MHC-I molecules on all body cells, they can recognize the same antigen at any body site. It is possible that keratinocytes express antigens that are identical or very similar to those of the tumor, known as antigen sharing or as molecular mimicry, respectively. [18] In our point of view, antigen sharing can presently be considered the most likely cause, as lung SCCs and keratinocytes both produce similar proteins including several cytokeratins. [19] In contrast, the histology of skin rashes of AC patients showed inflammation with lymphocytes located predominantly below the basal cell membrane within the dermis, which contains glandular structures (e.g. eccrine sweat glands). Those may share antigens with lung ACs.

Limitation of the study was the small sample size of 40 patients. One needs to keep in mind that immunotherapy for treating NLCSCs has only recently been approved and was provided through the early access program. We are confident that the recent approval now enables broader access to immunotherapy, which will allow our findings to be compared to future investigations from larger sample sizes.

In conclusion, our results suggest that the tissue tropism of lymphocytes in skin irAEs may be more specific than previously known, opening new opportunities for elucidating the underlying molecular mechanisms.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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Table 1. Patient characteristics.

Parameter			
Patient number			40
Age (years)		Mean	65.5
		Range	46 - 88
Gender		Male	22
		Female	18
Histological type		Adenocarcinoma	23
		Squamous cell carcinoma	14
		Mixed type	3
Nivolumab cycles		Mean	7
		Range	1 - 25
Nivolumab cycles until adverse skin reaction		Mean	3
		Range	2 - 8
Type of adverse skin reaction		Plaques	4
		Pruritus	3
Tumor response in patients with adverse skin reaction		Response	5
		No response	2

Table 2. Clinical presentation and histology of 4 patients

Patient	Gender, age in years	Onset (after cycles/days)	NSCLC[a]	Clinical presentation	Histology
1	m; 69	2/15	SCC[b]	Focal plaques with thick squamae on the extremities, with burning sensation, no mucosal involvement	Lichenoid dermatitis with interface-component at the dermo-epidermal junction and CD8+ cell infiltrate in the epidermis and dermis.
2	m; 68	2/37	SCC	Focal plaques with thick squamae on the	Lichenoid dermatitis with interface-component

				extremities, mild pruritus and burning, no mucosal involvement	at the dermo-epidermal junction. CD8+ cell infiltrate visible in the epidermis, with apoptotic keratinocytes, and the dermis.
3	f; 86	1/7	AC[c]	Focal scaly plaques on the extremities with pruritus, no mucosal involvement	Spongiotic dermatitis with accumulation of CD8+ T-cells below the basal cell membrane, sparse

					affection of epidermis.
4	m; 59	8/111	AC	Focal erythematous plaques with fine scaling on the upper face, no mucosal involvement	Lymphocytic infiltrate with accentuation in the deep dermis. Sparse interface component with sporadic vacuolization of basal keratinocytes.

[a]NSCLC: non-small cell lung cancer

[b]AC: adenocarcinoma

[c]SCC: squamous cell carcinoma

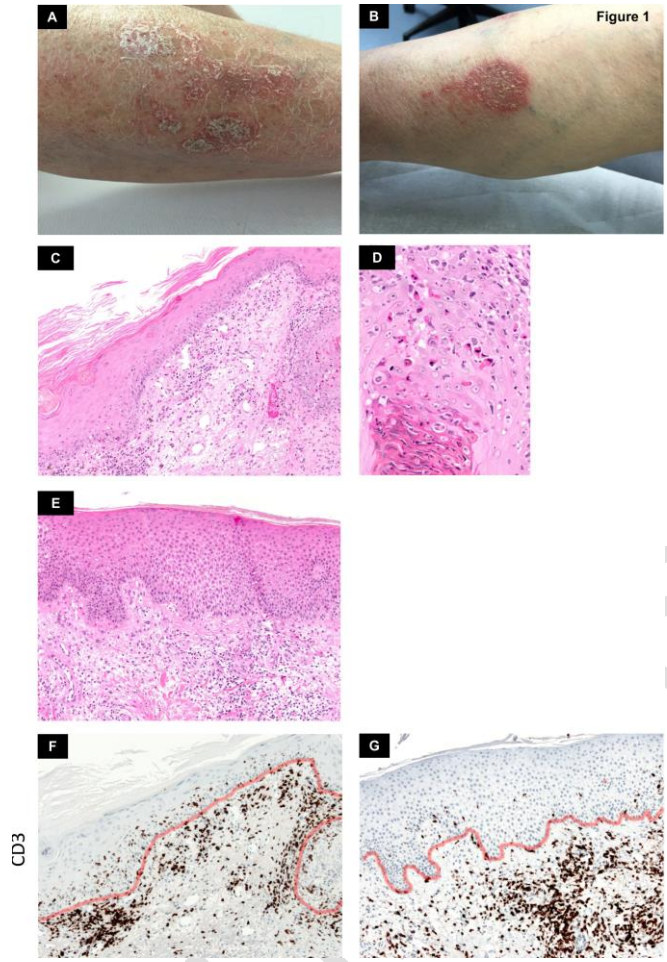

Figure 1. a. Skin rash under nivolumab in a patient with squamous cell carcinoma (SCC) of the lung or **b.** adenocarcinoma (AC) of the lung. **c.** Histopathology shows an inflammatory infiltrate migrating into the epidermis in SCC patients (hematoxylin eosin (HE), 100x). **d.** Apoptotic keratinocytes, also called Civatte bodies, are visible in SCC patients (HE, 200x). **e.** The infiltrate does not seem to migrate in AC patients (HE, 100x). **f.** Immunohistochemistry shows that the inflammation consists mainly of lymphocytes in both SCC (CD3, 100x) and **g.** AC (CD3, 100x) patients. The basal cell membrane is outlined in red.

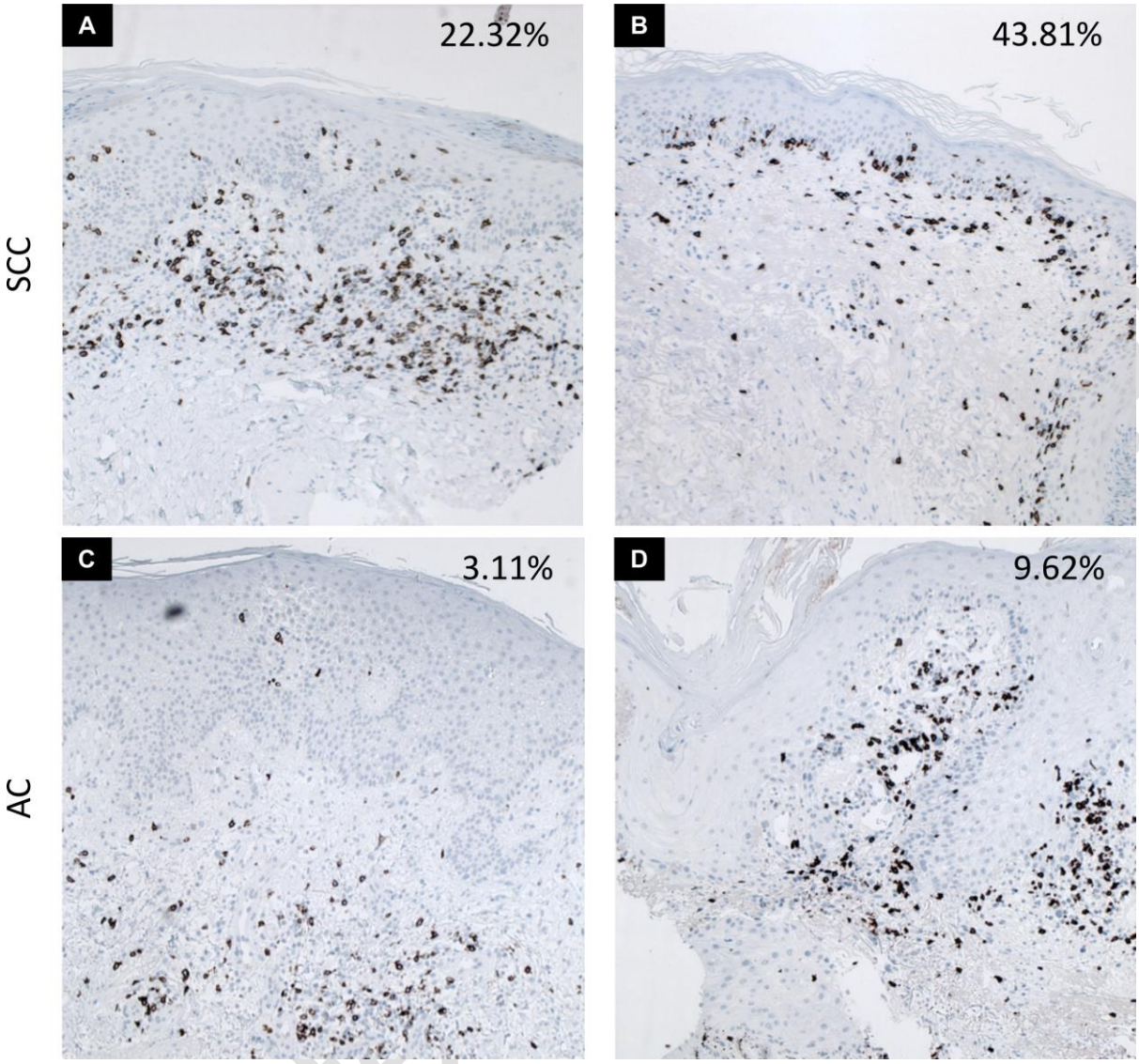

Figure 2. a.b. Immunohistochemistry with a CD8 stain of skin rash of 2 SCC patients (CD8, 100x) and **c.d.** 2 AC patients (CD8, 100x). The migration of the inflammation appears to differ among tumor subtypes. The number in the upper right corner of each panel indicates the percentage of CD8+ T-cells in the epidermis compared to the total of CD8+ T-cells of 4 high-power fields. They illustrate that the relative CD8+ T-cell count in the epidermis is greater among 2 SCC patients than among 2 AC patients.

Verdankung

To my family, friends and those who have supported me, guided me and pushed me to perform.

Thank you.

# Begleittext zur Publikation (equal contribution)

Von Omar Hasan Ali

Background

The clinical application of checkpoint inhibitors (CIs), or immunotherapy, marks a fundamental advancement in cancer therapy and management.[1] Prospective trials with large-scale cohorts comparing treatment of NSCLC with CIs to chemotherapy have shown that CIs result in better progression free survival and overall survival.[2-4] Data also suggests that progression-free survival can be sustained over years.[4]

Cytotoxic T-cells hold a crucial role in recognizing and fighting malignant cells. However, they need to be susceptible to modulation to prevent them from exercising autoimmunity. One way of modulation is the expression of surface receptors that mediate their exhaustion and in effect suppress their activity. Examples of such receptors are cytotoxic T-lymphocyte-associated Protein 4 (CTLA-4) and Programmed Cell Death Protein 1 (PD-1). Some cancers, such as melanoma and non-small cell lung cancer (NSCLC), are capable of exploiting these modulatory mechanisms by producing ligands that cause T-cell suppression.
T-cell activation requires two signals: first, binding of an antigen-MHC-complex to the surface T-cell receptor (TCR). Secondly, binding of ligands to a co-receptor, such as CD28. CD28 is responsible for early TCR-signaling amplification. It is counteracted by CTLA-4, a suppressive T-cell surface receptor that binds the same ligand as CD28. In conclusion, blocking CTLA-4 with the antibody ipilimumab promotes the ligand binding to CD28 and thus endogenous antitumor response. PD-1 is another example of a T-cell suppressing surface receptor and commonly found in peripheral tissues, thus causing an on-site suppression.[5]

Despite these seemingly obvious mechanism, the tumor response rate to CI treatment is reportedly around 40%, even in the group of PD-L1 positive NSCLC only for reasons not yet fully understood.[4]
Validated predictive factors for tumor response are still scarce and under investigation. In melanoma treatment, favorable baseline values seem to be a high relative Eosinophil count, high lymphocyte count, low LDH and no metastases other than soft tissue metastases.[6] In NSCLC patients the response rate among patients with PD-L1 producing tumors is higher compared to those not producing PD-L1.[4] A general association with cancer PD-L1 production and better response, however, remains controversial since it seems to vary among tumor types.[7]

In Switzerland, ipilimumab (anti-CTLA-4), pembrolizumab and nivolumab (both anti-PD1) are admitted as treatment options in metastasized melanoma and nivolumab for second-line treatment of NSCLC.

Since CIs increase immune system activity, they lead to immune-related adverse events (irAEs). Examples are colitis, endocrine disorders and skin disorders. Common skin irAEs are pruritus and rash.[8] Among all organs the skin is most commonly affected [9] and in case of anti-CTLA-4 skin irAEs appear earliest compared to other organs.[10] Fortunately, skin irAEs tend to be mild, usually reaching a severity on the Common Toxicology Criteria of Adverse Events (CTCAE) of 1-2.[11] Low-severity skin irAEs are well manageable with topical corticosteroids, rarely necessitating a break from therapy. In the rare case of skin irAEs of CTCAE grades 3-4 a pause of therapy and intervention with systemic immunosuppressants, such as corticosteroids, are recommended.[12]

In melanoma patients under immunotherapy, the histology of skin rashes has been investigated. Most of them were characterized by a lichenoid inflammation pattern, which suggests involvement of cytotoxic T-cells.[13, 14] The appearance of vitiligo during CI treatment of melanoma is also regarded a skin irAE. Developing vitiligo has been attributed to antigen sharing: T-cells identify and target antigens in melanoma that may also naturally occur in normal melanocytes.[15] Therefore, vitiligo development in melanoma patients may be a surrogate parameter for a favorable immune system reaction against melanoma antigens and may thus be associated with a better outcome.[16, 17]

To the knowledge of the authors, a systematic histopathologic characterization of skin rashes in a cohort of NSCLC patients under CI-therapy has not yet been published.

Hypothesis

NSCLCs can roughly be subdivided into two subtypes: squamous NSCLC (sNSCLC) and adenocarcinoma-type NSCLC (aNSCLC).[18] This made skin rashes induced by CIs for NSCLC treatment suitable candidates for exploring the hypothesis, that skin rashes may be caused by sharing autoantigens in tumor and healthy tissue. sNSCLC mostly likely contains antigens that are found in the epidermis. Therefore, the authors expected an inflammatory infiltrate consisting of $CD8^+$ T-Cells in the epidermis. aNSCLC, on the other hand, most likely carries antigens found in the dermis since this is where glandular structures are rooted.[19] The authors hypothesized that skin rashes in patients with aNSCLC T-cells will more likely be found below the basement membrane in the dermis.

The purpose of this study was to

1. characterize skin irAEs in NSCLC patients treated with nivolumab
2. assess if the occurrence of skin irAEs correlated with disease progression and
3. to examine histological patterns of inflammation in sNSCLC and aNSCLC to elaborate on the possibility of a different T-cell distribution.

Conclusions

We observed epidermotropism of cytotoxic $CD8^+$ T-cells into the epidermis in 2 patients with sNSCLCs. This effect was not observed in 2 patients with aNSCLCs. This supports the thesis that antigen sharing in skin and tumor could be crucial in developing skin irAEs, which are rather collateral damage than toxicity. Characterization of skin irAEs and relation to survival can be read in the “Results” section of the paper.

Author contributions

Management: Study coordination was supervised by Prof. Dr. Lukas Flatz (LF). Application for the local Ethics committee was written by Dr. Omar Hasan Ali (OHA) and reviewed/edited by Dr. Stefan Diem (SD) and LF. Lists of patients treated with nivolumab for NSCLC were provided by SD and Dr. Martin Früh. OHA and LF performed the skin biopsies at the Department for Dermatology, Venerology and Allergology at the Cantonal Hospital of St. Gallen. OHA extracted and summarized all published clinical parameters of the patient cohort (age, histologic NSCLC subtype, number of treatments given and disease progression according to the radiology reports based on RECIST 1.1 criteria). He further recorded skin irAEs with type, onset, duration and treatment. All parameters were retrospectively collected sourcing from the KSSGs electronic patient management system and paper files. Summarizing of results was done by OHA, while interpretation of the results was done collectively by OHA, SD and LF.

Tables and figures: All tables and figures of this study were designed by OHA, SD and LF. Histology slides including hematoxylin and eosin staining as well as CD3, CD4 and CD8 immunohistochemistry were initially assessed and provided by Dr. Eva Markert (EM). Slides were provided by Prof. Dr. Wolfram Jochum. Assessment of histology was done by EM and LF. T-cell distribution assessment and recording was performed by OHA using the high-powerfield technique. The resulting distributions, as displayed in Figure 2, were individually reviewed by LF and EM. Clinical pictures were provided by OHA and LF.

Manuscript: The manuscript was written by OHA, SD and LF. Important scientifc input and data interpretation was provided by Prof. Lars E. French, Dr. Katrin Kerl and Prof. Dr. Daniel E. Speiser.

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